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Environmental Friendly Synthesis, Characterization of Surface Modifiable Starch Encapsulated Gold Nanoparticles with Bactericidal and Catalytic Activity

Dillon Pender
Western Kentucky University, dillon.pender472@topper.wku.edu

ENVIRONMENTAL FRIENDLY SYNTHESIS, CHARACTERIZATION OF SURFACE MODIFIABLE STARCH ENCAPSULATED GOLD NANOPARTICLES WITH BACTERICIDAL AND CATALYTIC ACTIVITY

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By

Dillon S. Pender

* * * * *

Western Kentucky University

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CE/T Committee:

Professor Rajalingam Dakshinamurthy, Advisor

Professor Kenneth Crawford

Professor Leslie Baylis

Approved by

Advisor
Department of Chemistry

ABSTRACT

In the present study, we report the green synthesis of gold nanoparticles (GNPs), using potato starch as a reducing as well as a stabilizing agent in an aqueous buffer medium. The resulting starch-GNPs (St-GNPs) were near spherical in shape with an average diameter of 15 ± 5 nm. In this approach, St-GNPs were characterized and investigated for their antibacterial properties against both gram negative (*Escherichia coli*) and gram positive (*Staphylococcus epidermidis*) bacteria. These St-GNPs were found to possess significant dose dependent antibacterial activity against bacteria. Also, St-GNPs showed a significant catalytic activity and can easily be functionalized using a simple, greener method for various applications in non-polar solvent. The overall results suggest the synthesis of multifunctional St-GNPs, which can be used as an antibacterial agent, catalyst and for a wide variety of environmental, biomedical, and industrial chemical applications.

Key words: Starch, green synthesis, multifunctional gold nanoparticles, antibacterial activity

Dedicated to my family & friends

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This project would not have been possible without the help, knowledge and support of so many people. I would like to first thank my advisor Dr. Rajalingam Dakshinamurthy for all of his insightful knowledge and unwavering encouragement.

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VITA

September 20th, 1991…….....Born – Elizabethtown, KY

2009…….Central Hardin High School,
Elizabethtown, KY

2011……Elizabethtown Community & Technical
College Graduate

Fall 2011……..KAS Undergraduate Chemistry
Department Oral Presentation Winner

Spring 2012…….Oral Presentation: ACS National Meeting
San Diego, California

FIELDS OF STUDY

Major Field: Chemistry & Biology

Minor Field: Biophysics

Concentration: Pre-medicine

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CHAPTER 1

INTRODUCTION

Increasing appearance of antibiotic-resistant strains of pathogenic bacterial species, in a short period of time is a major public health concern (1,2). Many commonly used traditional antibiotics are no longer effective and this situation is aggravated by a decline in the development of new antibiotics (3). To fight against these challenges advanced therapeutic strategies are required; among all the advanced strategies nanomedicine is considered as one of the promising approaches (4-6).

Nanomedicine is considered to have a potential for major improvements in health care (7). This potential therapeutic activity is due to the very small size of nanoparticles which offer the ability to interact with complex biological macromolecules. Thus, nanomedicine creates enormous opportunities of novel applications in medicine such as diagnostic, antibacterial activity, medical imaging, targeted drug delivery and immune therapy (8). But with its advancement, nanotechnology focuses on the synthesis of environmentally friendly, biodegradable and multipurpose nanoparticles (9). Achieving the above goals are limited because of the following reasons: a) use of non-ecofriendly synthesis method, b) complication in method of synthesis, c) uncontrolled disparity of nanoparticles, d) non-biocompatibility, e) non-convenient surface bioconjugation, f) irregular sizes and g) lack of stability (10-12).

Considering the above limitations, GNPs have been employed most extensively because of some of its properties such as stability, rigidity, non- toxic nature, remarkable plasmon-resonant optical properties, biocompatibility, imaging ability and easy multi functionality. Owing to above properties the GNPs are useful for a wide variety of environmental, biomedical and industrial chemical applications (13-15).

In this study, we report the synthesis of multifunctional GNPs using potato starch (obtained from *Solanum tuberosum)* as the reducing and stabilizing agent. Starch is a natural, renewable and biodegradable polymer produced by many plants as a source of energy (16). It is the second most abundant biomass in nature and the most common carbohydrate in the human diet (17). Chemically, the starch molecule is a polysaccharide assembled from simple glucose molecules; it can contain five hundred to several hundred thousand glucose molecules joined by glycosidic linkages into a single structure (18).

The synthesis method employed is completely green, single step, single phase without addition of any external secondary capping agent. The starch encapsulated gold nanoparticles (St-GNPs) obtained were near spherical in shape with an average diameter of 15 ± 5 nm. Here we explored the three important applications of St-GNPs: First we tested the antibacterial activity of the St-GNPs against both gram negative (*E. coli*) as well as gram positive (*Staphylococcus epidermidis*) bacteria and found the St-GNPs to possess significant size dependent antibacterial activity. The catalytic activity studies explored the application in environmental safety by removing *p*-nitrophenol from the environment. The functionalization of St-GNPs with organic ligand leads to solubility in non-polar solvents for applications in non-aqueous chemistry. In brief, we report the

synthesis of multipurpose St-GNPs, find application in the areas of nanomedicine, environmental safety (catalysis) and non-aqueous chemistry.

CHAPTER 2

MATERIALS AND METHODS

2.1. Reagents and material:

Chemicals including $KAuCl_4/HAuCl_4$, Lysogeny Broth (LB) agar, and tryptic soy agar were purchased from Aldrich, St. Louis, MO. *Escherichia coli (E. coli)* and *Staphylococcus epidermidis (S. epidermidis)* were either purchased from Invitrogen, Carlsbad, CA or obtained from the biological specimen collection at Western Kentucky University. Analytical grade chemicals were typically used. Potatoes (*Solanum tuberosum*) were purchased from local markets.

2.2. Extraction of starch from potato:

Starch was extracted from potatoes by a liquid-solid extraction process using water as a solvent. A medium sized potato was selected and washed thoroughly several times to remove the soil contents from its surface. The washed potato was then peeled, cut into small pieces and ground into a slurry using a Waring laboratory blender with the addition of ice cold nanopure water. The potato slurry was placed in a strainer to separate the water (containing starch) from the fruit pulp. The leftover pulp was pressed and washed with water for several times to extract the traces of starch. The extracted starch was allowed to settle down at room temperature and then the supernatant (water) was decanted. Further, the starch solution was treated with 0.1 M NaOH solution to dissolve

the less soluble impurities such as proteins and other complex structures. The NaOH was removed by several washes of water and the pure starch so obtained was then allowed to dry in a desiccator (17).

2.3. Synthesis of St-GNPs:

St-GNPs were synthesized by the reduction of Au^{+3} ions using starch in an environmentally friendly buffer solution (pH ~ 7.2 ± 0.2). For a typical synthesis, 1.5 mM $KAuCl_4$ was mixed with buffer containing 2 mM starch solution. The mixture was incubated in an orbital shaker at the speed of 150 rpm for 12 hours. Change in the color of solution from white to yellow indicates the reduction of Au^{+3} to Au^0 i.e., the formation of St-GNPs. To collect the nanoparticles, samples were centrifuged at 12000 rpm (14862g) for 20 min, and the supernatant was discarded. The precipitate was thoroughly washed with nanopure water several times and used for further analysis.

2.4. Characterization of St-GNPs:

To analyze the surface plasmon resonance, the absorption spectra of the St-GNPs was measured using PerkinElmer's LAMBDA 35 UV/vis spectrophotometer. The size and shape of the synthesized St-GNPs was investigated using a transmission electron microscope (JEOL-TEM). Samples for the TEM images were prepared by placing a drop of St-GNPs solution onto a 400 mesh size Formvar coated copper grid and air-drying them for 5 minutes. Excess water was removed by using a filter paper wedge. The elemental composition of the nanoparticles was determined by energy dispersive x-ray spectroscopy (EDS) using JEOL-JSM-S400 LV with IXRF system. Samples for EDS were prepared by placing a thick slurry of St-GNPs onto an aluminum stub and allowing

the samples to dry in a desiccator. The nature of interaction of starch with the GNPs was analyzed by FTIR in transmittance mode using Perkin-Elmer Spectrum 100 FT-IR spectrometer at 4 cm^{-1} resolution.

2.5. Determination of antibacterial activity of St-GNPs:

Spread Plate Technique is a primary screening test that was used to investigate the effect of St-GNPs on the viability of bacteria (23). St-GNPs of various concentrations (0.3 mg mL^{-1}, 0.6 mg mL^{-1}, 1.2 mg mL^{-1}, 2.4 mg mL^{-1}, 4.8 mg mL^{-1}) were inoculated with 10^9 CFU/cm^3 in a series of test tubes containing 4 mL of sterile liquid media. This dilution allowed for ~400 bacterial colonies to be formed on the control. A control was made by inoculating the media with *E. coli* / *S. epidermidis* in absence of St-GNPs. All tubes were incubated at 37 ºC for 12 hours. After 12 hours, 10 µL of the bacterial suspension (*E. coli* / *S. epidermidis)* obtained from St-GNPs treated and control sample were cultured on fresh LB agar / soy tryptic agar plates. These plates were then incubated for 12 hours at 37 ºC and counted for number of colonies (19,20). This process was repeated thrice in order to obtain reproducible results.

2.6. Morphology of bacteria treated with St-GNPs:

To determine the morphology of the bacteria treated with St-GNPs, ultra-thin cross-sections of bacterial cells were observed through TEM. Various concentrations of St-GNPs were inoculated with 10^9 CFU/cm^3 *(E. coli* / *S. epidermidis)* in a series of test tubes containing 4 mL of sterile liquid media. Controls were made by inoculating the media with *E. coli* / *S. epidermidis* in absence of St-GNPs. All tubes were incubated at 37 ºC for 12 hours. 1 mL of sample was collected at different time points from the test tubes

and centrifuged at 6000 rpm for 3 minutes. All the samples were thoroughly washed with nanopure water and then placed on Formvar coated 400 mesh size copper grids and observed under JEOL-TEM. For cross-section analysis, bacterial cell cultures were fixed using 16 % w/v paraformaldehyde and 10 % w/v gluteraldehyde as primary fixing agents in 50 mM sodium cacodylate buffer (pH ~ 7.4) and incubated for 2 hours in the hood. The fixed samples were washed thoroughly using the buffer solution and post fixed using 1 % OsO_4 and incubated for 1 hour at 25º C. The post-fixed samples were then thoroughly washed with nanopure water followed by the treatment of graded ethanol series (25, 50, 75, 95 and 100 %) for 5-10 minutes. The dehydrated samples were further infiltrated by incubation in a graded series of Spur's epoxy resin (33, 66, 95 and 100 %) for 1 hour and left overnight in a fresh 100 % resin. The samples were centrifuged through fresh resin in BEEM capsules and hardened at 70 °C for 18 hour. Ultra-thin sections of the pelleted samples were cut on an RMC MT-X ultra-microtome using glass knives. Sections were stained with 2 % aqueous uranyl acetate and Reynold's lead citrate for 15 minutes and 3 minutes respectively and examined using a JEOL-100CX TEM.

2.7. Fluorescence assay:

To determine the permeability of the bacterial cell wall, bacteria treated with St-GNPs were incubated with a fluorescence dye propidium iodide (PI) (4). PI is a nucleic acid binding dye which is impermeable through normal, healthy bacterial cell wall. Hence by treating the cells with PI the proportions of living and non-living cells can be determined on the basis of fluorescence observed. Both *E. coli* and *S. epidermidis* were cultured in liquid media in the presence of different concentrations of St-GNPs. The

samples were collected and centrifuged at 6000 rpm for 3 minutes and washed twice with PBS buffer (pH ~ 7.2). These bacterial cells were incubated with 10 mM PI in a dark environment for 30 minutes at room temperature. For sampling, 5 µL of the bacterial suspension was placed on a glass slide, mounted with a cover slip and observed under a fluorescence microscope. The bacterial suspension without St-GNPs was used as a control.

2.8. Catalytic activity:

The catalytic activity of St-GNPs was monitored by following the reduction kinetics of p-nitrophenol using a UV/vis spectrophotometer (23). For a typical reaction, 250 µL of 20 mM $NaBH_4$ solution was added to 200 µL of 0.5 mM p-nitrophenol solution. The absorption peak at 400 nm was monitored using PerkinElmer's LAMBDA 35 UV/vis spectrophotometer. Upon addition of 200 µL of St-GNPs to the reduction mixture a quick change in color from light yellow to green-yellow with a red shift from 315 to 400 nm occurred. The resulting time dependent spectrum was recorded at 400 nm wavelength.

2.9. Surface modification:

Phase transfer of the St-GNPs from aqueous to organic phase was performed by modifying its surface using Dodecanethiol (DDT) (24). Synthesized St-GNPs were washed several times and dispersed in sterile nanopure water. To this aqueous St-GNPs solution, a mixture of DDT and hexane was added. The resultant mixture was shaken vigorously until the St-GNPs surface was capped with DDT and extracted into the organic phase.

CHAPTER 3

RESULTS AND DISCUSSION

Fabrication of multifunctional GNPs by a completely green synthesis method is a need as well as a challenge for current nanotechnology. In this study, we have provided a simple and completely green method to synthesize GNPs. Reaction ingredients like potato starch and aqueous buffer are not only eco-friendly in nature, but also easily biodegradable.

3.1. Synthesis and characterization of St-GNPs:

St-GNPs were synthesized by adding various concentrations of starch (0.25, 0.5, 1, 2 and 3 mM) to an aqueous solution of $KAuCl_4$ (1.5 mM). Samples were incubated at 37 ºC for 12 hours in an incubator shaker under normal atmospheric pressure. After 4 hours, the reaction mixture showed a change in color from light yellow to darker yellow, indicating the complete reduction of Au^{3+} to Au^0. Among the various concentrations, the optimum starch concentration for the formation of nanoparticles with consistent size, shape and disparity was found to be 2 mM. Any further decrease or increase in concentration caused either immature nuclei or cluster formation. The synthesized St-GNPs were examined using UV/vis spectroscopy. It is known that GNPs exhibit the

localized surface plasmon resonance phenomenon, which is evidenced by an absorption band in the visible region of the optical spectrum (25,26). The synthesized St-GNPs showed a plasmon resonance peak at 540 nm, which can be attributed to the presence of ~15 nm St-GNPs "**Figure 1A**". The absence of absorbance at wavelengths greater than 600 nm indicates that the St-GNPs are in a well dispersed state in solution (27). To confirm the morphology, the samples were observed under TEM, which showed particles that were spherical in shape with an average diameter of 15 ± 5 nm "**Figure 1B**".

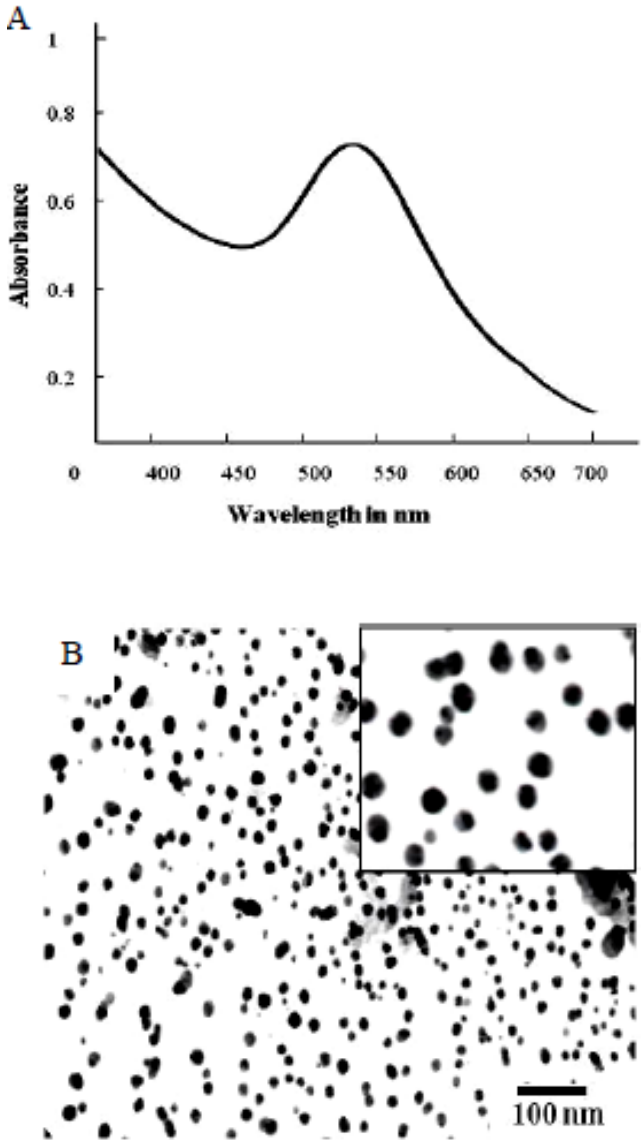

Figure 1. (A) UV/ vis spectra for St-GNPs with absorbance peak at ~ 540 nm. (B) TEM image of St-GNPs with average diameter 15 ± 5 nm;

Energy dispersive spectrum (EDS) was used to determine the elemental composition of the St-GNPs. A thoroughly washed St-GNPs sample was used for area profile analysis. The results showed a strong peak for gold at 2.18 KeV which is the characteristic feature of metallic gold. A peak for carbon was also found indicating the encapsulation of the starch on the surface of GNPs as shown in "**Figure 2A**". To determine the interaction of the starch with the St-GNPs, the samples were further analyzed using FTIR. Results suggested a significant difference between the spectra for the control (starch solution) and St-GNPs. The broadening of the band around 3100 – 3400 cm^{-1}, which corresponds to hydroxyl functional group, indicates the electrostatic interaction between starch and surface of the GNP "**Figure 2B**" (28).

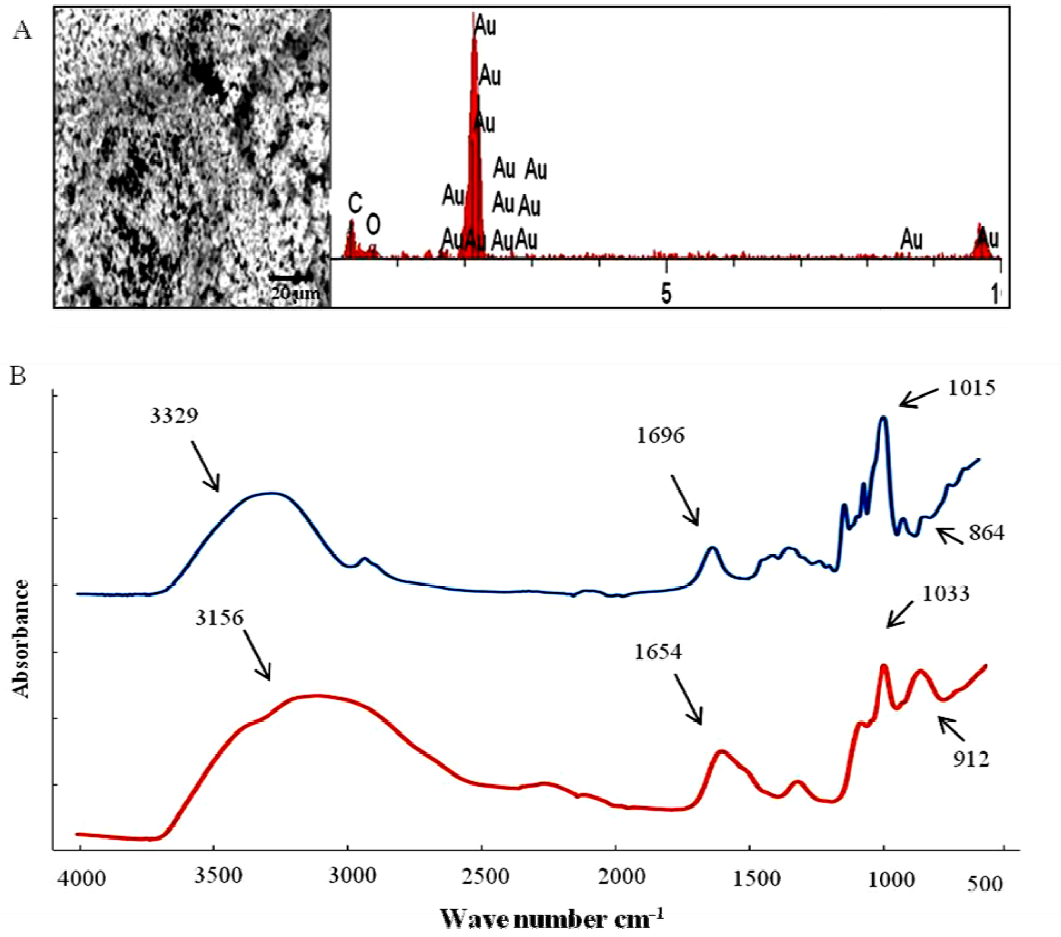

Figure 2. (A) EDS of starch GNPs showing elemental composition with 80% of gold, 15% of carbon and 5% of other trace elements, which indicates the starch encapsulation on the gold nanoparticles; (B) Interaction of starch molecule with metallic nanoparticle surface evident by FTIR: control spectrum of pure potato starch (blue), spectrum for starch encapsulated gold nanoparticles (red).

3.2. Antibacterial activity:

Nanomedicine is considered as a new hope for future medicine and is considered to have a potential for major improvements in health care. One of the growing areas of nanomedicine involves the fabrication of antibiotics. Interestingly, functionalized GNPs have been considered to be more efficient antibiotics as compared to the other traditional antibiotics. In this context, we evaluated antibacterial properties of St-GNPs through solid plate based assays and evaluated the mechanism of antibacterial action by electron microscopy and fluorescence microscopy.

Spread plate technique was employed to determine the number of viable cells recovered after treating with different doses of St-GNPs. In this experiment, both gram-negative and positive bacterial strains (*E. coli* and *S. epidermidis*) were treated with increasing doses of St-GNPs in sterile liquid media. After the time at which the control reached its stationary phase (i.e., 12 hours), all the samples were diluted 10^{-5} times in a fresh media and cultured in a solid agar plate. Upon complete incubation (12 hours), the number of colonies was counted and the graphs for number of colonies versus the increasing doses of St-GNPs were plotted. The result for both the strains indicated the consistent decrease in number of recovered viable bacterial cells with increasing doses of nanoparticles. The minimum inhibitory concentration (MIC) for *E. coli* was found to be 4.8 mg/mL which was more than the MIC obtained for *S. epidermidis* (1.2 mg/mL). Thus, *S. epidermidis* was found to be more antibacterial sensitive towards St-GNPs as compared to that of *E. coli* "**Figure 3 & 4**".

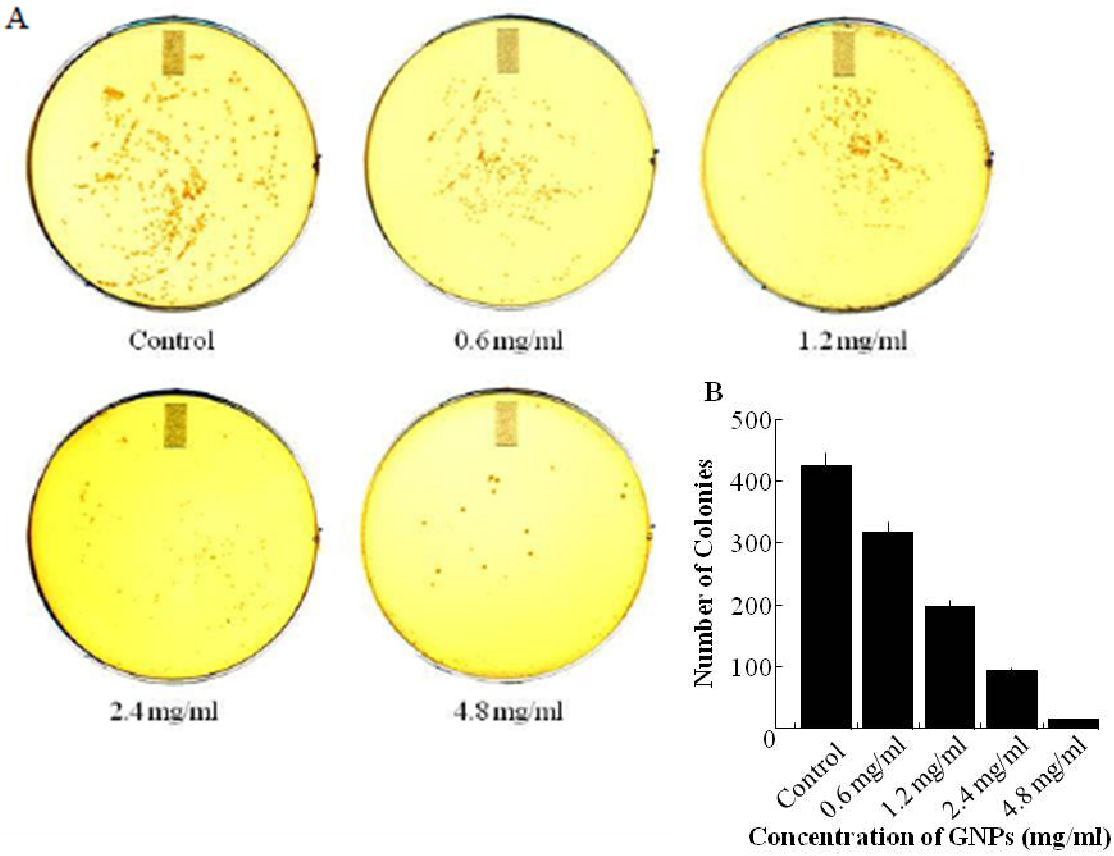

Figure 3. Spread plate assay for *E. coli* treated with different concentrations of St-GNPs (A) LB agar plates with control (untreated sample) and the St-GNPs treated samples showing the anti-bacterial effect; (B) Graph plotted with number of *E. coli* colonies grown on each LB agar plate against different concentrations of St-GNPs (mg/ml). Experiment was repeated thrice to confirm the validity of the trend.

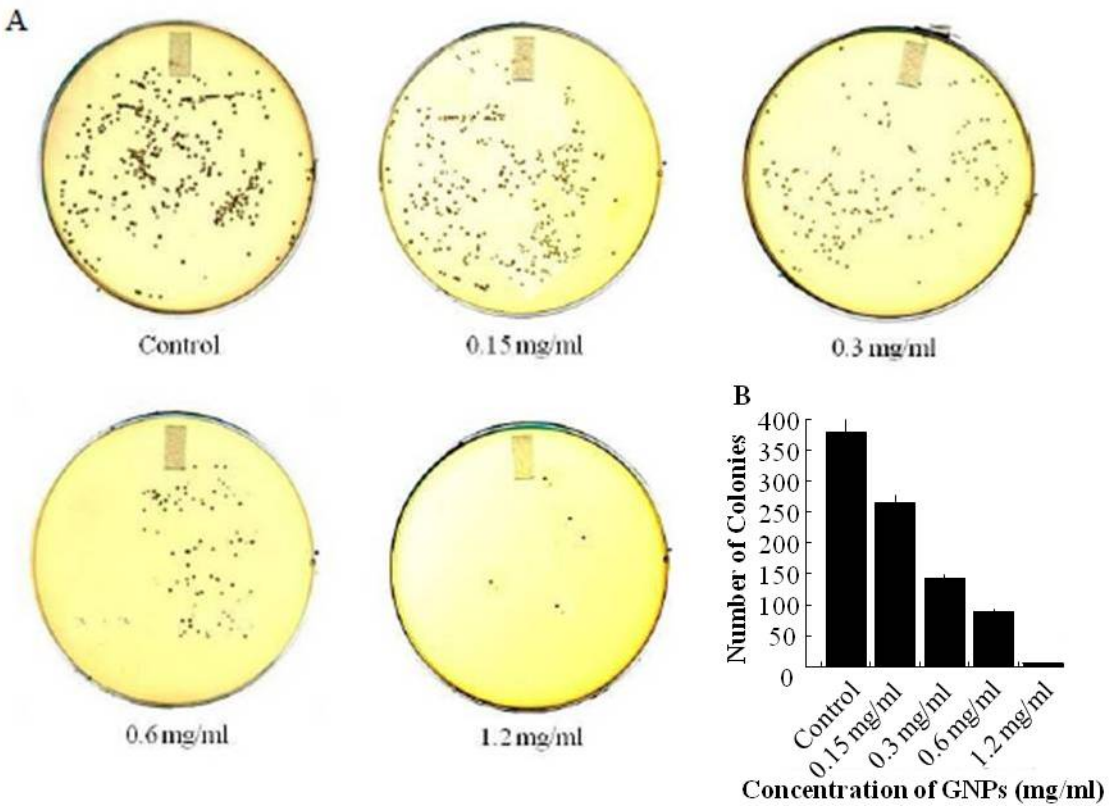

Figure 4. Spread plate assay for *S. epidermidis* treated with different concentrations of nanoparticles (A) Tryptic soy agar plates with control (untreated sample) and the treated samples showing the anti-bacterial effect; (B) Graph plotted with number of *S. epidermidis* colonies grown up on each Tryptic soy agar plate against different concentrations of St-GNPs (mg/ml). Experiment was repeated thrice to confirm the data trend validity.

The potency of St-GNPs, as an antibacterial agent was evaluated by the comparison with that of the standard antibiotic, kanamycin. Against *E. coli* the potency of the St-GNPs at their minimum inhibitory concentration (4.8 mg/mL) was found to be similar to that of kanamycin, which is 4.8 mg/mL. A similar comparison against S. *epidermidis* showed that the potency of St-GNPs (minimum inhibitory concentration of 1.2 mg/mL) to be greater than that of kanamycin, which is 2.4 mg/mL "**Figure 5**".

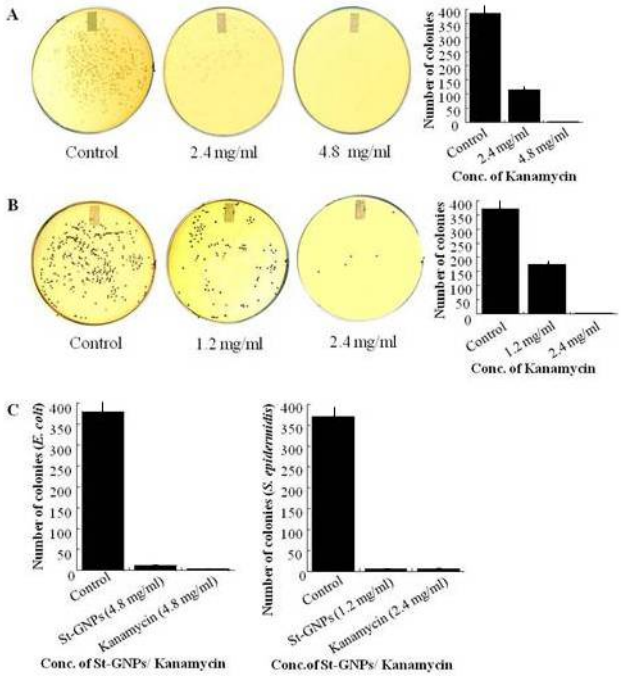

Figure 5: (A) Spread plate assay for *E. coli* treated with different concentrations of kanamycin. Images of plates with control (untreated sample) and the kanamycin treated samples showing the anti-bacterial effect; Graph plotted with number of *E. coli* colonies grown against different concentrations of Kanamycin (mg/ml). (B) Spread plate assay for *S. epidermidis* treated with different concentrations of kanamycin. Images of plates with control (untreated sample) and the kanamycin treated samples showing the anti-bacterial effect; Graph plotted with number of *S. epidermidis* colonies grown against different concentrations of Kanamycin (mg/ml). (C) Comparison graph plotted with number of *E. coli* colonies grown against St-GNPs/ kanamycin (mg/ml). Comparison graph plotted with number of *S. epidermidis* colonies grown against St-GNPs/ kanamycin (mg/ml). Experiment was repeated thrice to confirm the data trend validity.

The composition of the bacterial cell wall is a possible key to explain the difference in antibacterial sensitivity of gram negative and gram positive bacteria (29,30). The cell wall of gram negative bacteria consists of multiple layers; chiefly consist of lipopolysaccharide, phospholipids and protein molecules. It also contains a thin peptidoglycan layer as an innermost layer. The combination of these layers provides an effective resistant barrier against nanoparticles (4,31,32). On the other hand the cell wall of gram positive bacteria mainly consists of several layers of peptidoglycan, which is unable to resist the nanoparticle action. Thus, this difference in the cellular wall component caused the decrease sensitivity of *E. coli* (33,34).

Determining the mechanism of action of an antibacterial agent is one of the important factors in exploring its proper usage and application. In this context, we studied the mechanism of action of St-GNPs by observing the changes occurred in the morphology of bacterial cells. This was done by taking cross-sections of the St-GNP treated bacterial cells. The samples for cross section were initially treated with St-GNPs in sterile liquid media and were collected at different time points. The TEM analysis for St-GNP treated *E. coli* cells showed significant morphological changes when compared to control (which did not show any significant morphological changes, even after 12 hours). At 0 minute, i.e. as soon as the St-GNPs were added to the *E. coli* culture, they were observed to be sticking onto the surface of bacterial cell wall. After 6 hours, the structural changes in the form of perforations or outer membrane vesicles (OMVs) were seen in the cell wall. Consequently, these structural changes resulted in complete membrane disruption near 12 hours "**Figure 6-I**". Membrane disruption leads to a reduced control over the movement of metabolites in and out of a bacterial cell. This

causes homostatic imbalance, leading to cellular metabolic discharge and ultimately death (35-37). The morphological studies of *S. epidermidis* cells also suggested the similar mechanism of bactericidal action. The gradual damage to the cell membrane was observed, eventually leading to complete disruption of the bacterial cell membrane. Thus, we assume the bactericidal action of St-GNPs through the disruption of bacterial cell membranes, leading to disturbed osmotic balance between the cell and its surroundings leading to cell death "**Figure 6-II**".

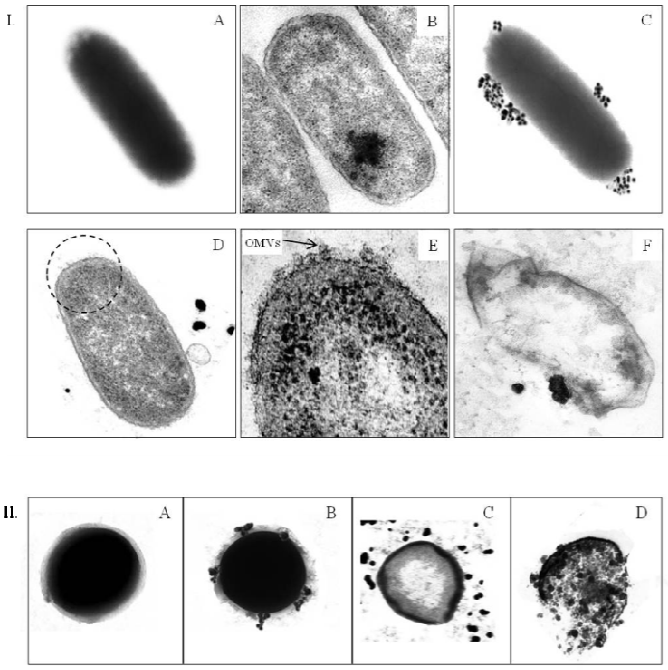

Figure 6. Visualizing St-GNP induced morphological changes in the bacterial cell. *Panel-I*- (A) TEM image of the normal *E. coli* cell at 0 hour; (B) Cross-section of normal *E. coli* cell; (C) TEM image of the *E. coli* cell treated with St-GNPs at 0 hour; (D) *E. coli* cell showing perforations/OMVs at 6 hours when treated with St-GNPs; (E) Magnification of the area showing perforation/OMVs in the 6 hours sample; (F) Lysed *E. coli* cell at 12 hours when treated with St-GNPs. *Panel-II*- (A) TEM image of the normal *S. epidermidis* cell at 0 hour; (B) TEM image of the *S. epidermidis* cell treated with St-GNPs at 0 hour; (C) *S. epidermidis* cell treated with St-GNPs at 6 hours; (D) Lysed *S. epidermidis* cell at 12 hours when treated with St-GNPs.

To further confirm the mechanism of anti-bacterial activity, fluorescence microscopy was performed. Propidium iodide (PI) dye was used to monitor the permeability of the cell membrane. PI is an intercalating agent and a fluorescent molecule which can be used to stain cells. PI binds to DNA as well as RNA and fluoresces red. PI is usually impermeable and cannot stain a normal healthy cell but PI is permeable to damaged or dead cells. Hence it is used to differentiate between living and non-living cells. The results clearly showed that the permeability of *E. coli* and *S. epidermidis* after treatment with St-GNPs for 12 hours was extensively more than the control (> 90 %) "**Figure 7-I & II**". This further confirms the antibacterial mechanism of action of St-GNPs through disruption of the bacterial cell wall.

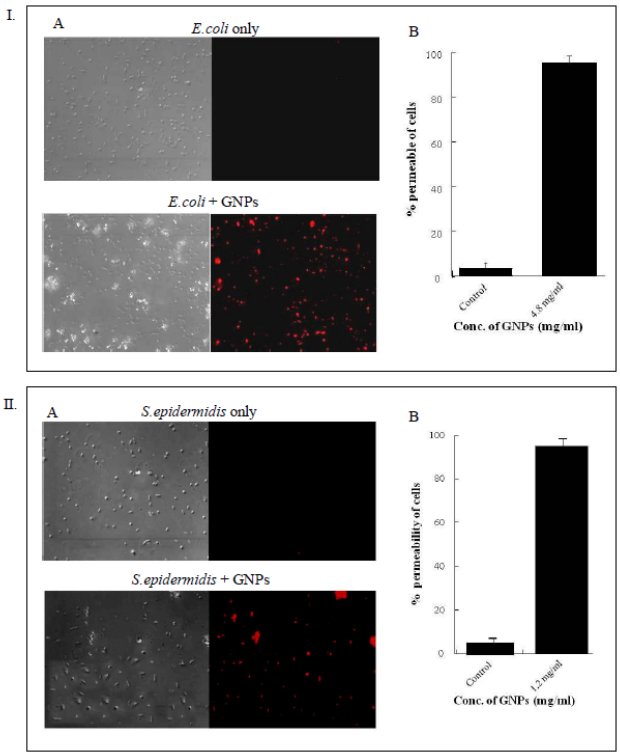

Figure 7. *Panel-I*- Monitoring St-GNPs induced permeability of *E. coli* cell membranes using propidium iodide dye. (A) On each image, the left half shows an image in the differential interference contrast mode, while the right half shows the corresponding fluorescence image; (B) Plot with percentage of permeable cells against concentration of St-GNPs. *Panel-II*- Monitoring St-GNPs induced permeability of *S. epidermidis* cell membranes using propidium iodide dye. (A) On each image, the left half shows an image in the differential interference contrast mode, while the right half shows the corresponding fluorescence image; (B) Plot with percentage of permeable cells against concentration of St-GNPs.

3.3. Catalytic activity:

Nitrophenols are industrial chemicals used to manufacture drugs, fungicides, insecticides, dyes and leather tanning. But most of the nitrophenols enter and pollute the environment, which can cause various blood disorders, headaches, drowsiness, nausea and cyanosis. Hence, nitrophenols are categorized as toxic environmental pollutants and current research focuses on their remediation (38). In this context, the catalytic activity of St-GNPs was demonstrated in the reduction reaction of *p*-nitrophenol by $NaBH_4$. An aqueous solution of *p*-nitrophenol shows an absorption peak at 317 nm and addition of $NaBH_4$ reduces the *p*-nitrophenol into nitrophenolate ion and hence the red shift to 400 nm (39,40). But, this process takes nearly 48 hours. Addition of St-GNPs resulted in instant conversion of nitrophenolate ions into aminophenol, which can be monitored by observing the fading of color due to a decrease in intensity of the absorption peak at 400 nm. A control experiment was performed with addition of starch powder to the *p*-nitrophenol and $NaBH_4$ mixture which did not show any change of absorption at 400 nm "**Figure 8**". In this context, pseudo-first order kinetics was used to evaluate the rate constant of the catalytic reaction. Owing to small size, St-GNPs showed a significant catalytic activity ($k\sim 3.4 \times 10^{-3} s^{-1}$). Thus the results clearly indicate the St-GNPs to be an efficient catalyst and useful agent for the removal of toxic pollutants like nitrophenols from the environment.

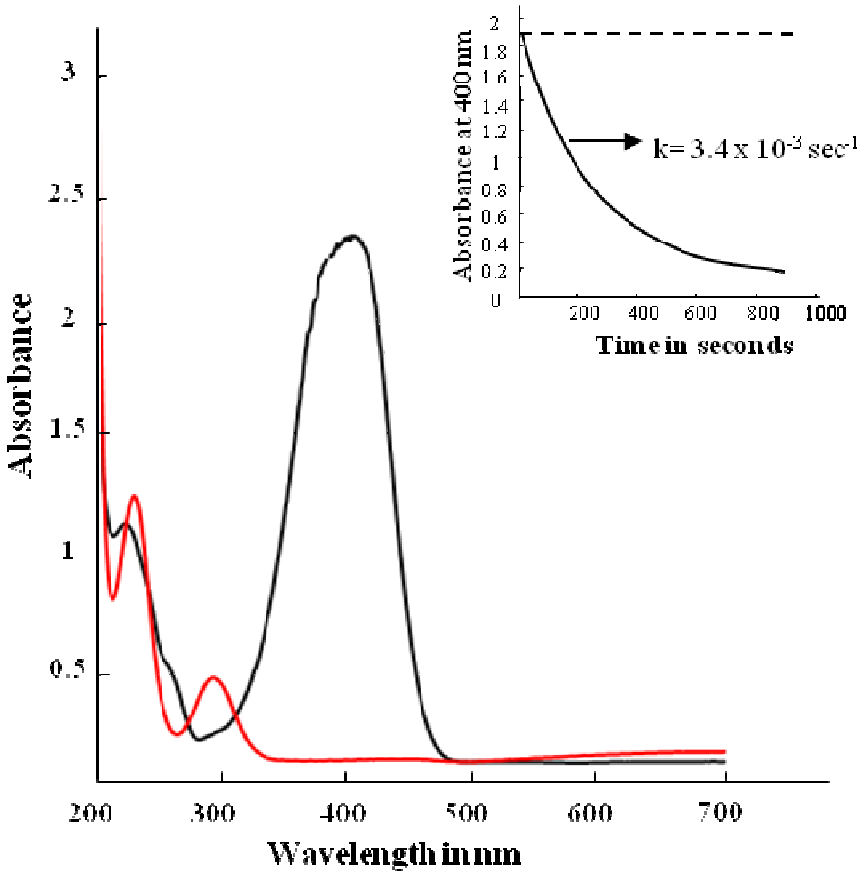

Figure 8. Absorption spectra, indicating the reduction of *p*-nitrophenol by $NaBH_4$, in the absence (black) and in the presence of St-GNPs (red). The inset shows absorption at 400 nm for *p*-nitrophenol (dotted) as a function of time without the presence of St-GNPs and the reduction of *p*-nitrophenol (solid) in the presence of St-GNPs as a catalyst.

3.4. Surface modification:

Multiple applications warrant nanoparticles with different types of ligands on their surface in non-aqueous solvents (41,42). Solely aqueous solubility of St-GNPs might limit its application to aqueous chemistry. But to explore its application into non-polar solvents, the nanoparticles were coated with 1-dodecanethiol. These nanoparticles were then phase-transferred to hexane simply by shaking a mixture of water and hexane "**Figure 9**".This method is simple, green, cheap and easy-to-adopt.

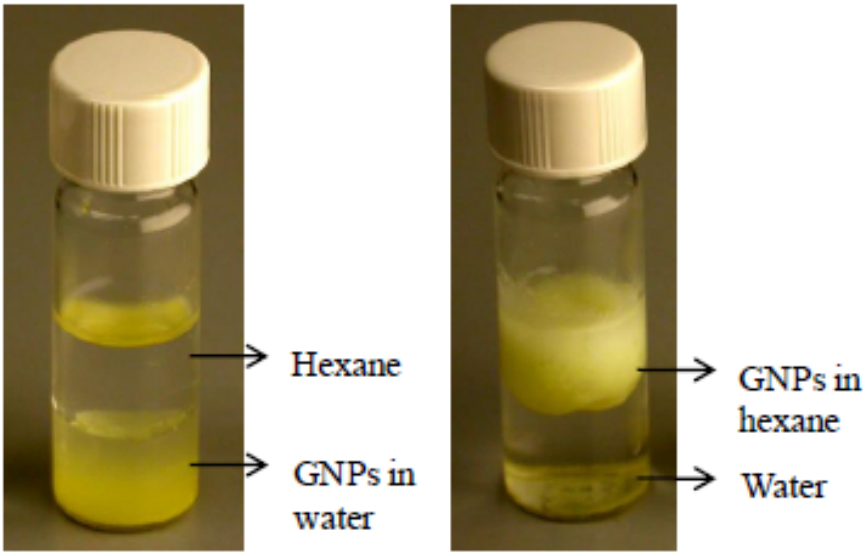

Figure 9. Functionalized St-GNPs with 1-dodecanethiol, to solubilize the nanoparticles in non-polar solvent. Digital image of St-GNPs suspended in water and hexane mixture, showing functionalized St-GNPs with 1-dodecanethiol suspended into hexane.

CHAPTER 4

CONCLUSION

In conclusion, we have reported the 'Green Synthesis' of near spherical shaped GNPs using potato starch as both the reducing as well as the capping agent. The synthesized St-GNPs were stable and biocompatible with a size range of 15 ± 5 nm. This method was environmentally friendly, single step/single phase without the use of any secondary capping agent and performed at room temperature and atmospheric pressure. These nanoparticles were shown to possess a dose dependent bactericidal activity. The anti-bacterial affect was due to the formation of perforations/OMVs which eventually lead to cellular destruction. We also reported the catalytic activity and the surface modification of St-GNPs which will enable a wide range of applications in the field of chemistry and biology.

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